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Application of *Rhizobium* sp. BHURC01 and Plant Growth Promoting Rhizobactria on Nodulation, Plant Biomass and Yields of Chickpea (*Cicer arietinum* L.)

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Abstract: Field-based experiments were conducted to evaluate the combined application of *Rhizobium* sp. BHURC01 and plant growth promoting rhizobacteria on chickpea production in two consecutive years. A positive influence of plant growth promoting rhizobactria and *Rhizobium* sp. BHURC01 on nodulation, plant biomass, nitrogen and phosphorus in nodule, grain and straw and yield related parameter were recorded in two year of field experiments. The maximum significant increase in nodule number, dry weight of nodule, root and shoot were recorded in co-inoculation of *Rhizobium* sp. BHURC01 and *Pseudomonas fluorescens* followed by co-inoculation of *Rhizobium* sp. BHURC01, *Azotobacter chroococcum* and *Bacillus megaterium* over uninoculated control in two year of field study while, nitrogen and phosphorus content increase in nodules, grain and straw. The *Rhizobium* sp. BHURC01 and *P. fluorescens* showed significant increase in all parameter due to more available nitrogen by *Rhizobium* sp. BHURC01 and more available of phosphorus, iron and plant hormones like indole-3-acetic acid (IAA) and antifungal activity by *P. fluorescens* in comparison of *B. megaterium* and *A. chroococcum*. Therefore, co-inoculation of *Rhizobium* sp. BHURC01 and *P. fluorescens* could be effective biofertilizer for chickpea (*Cicer arietinum* L.) production.

Key words: *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Bacillus megaterium*, co-inoculation, IAA, yield

INTRODUCTION

The plant rhizosphere is an important soil ecological environment for plant-microbe interactions. It involves colonization by a variety of micro-organisms in and around the roots which may result in symbiotic, associative, neutralistic or parasitic relations within the plant, depending on the type of microorganism, soil nutrient status and plant defense system and soil environment. Due to excessive use of the chemical fertilizers and plant protection chemicals, the rhizosphere microflora has been greatly affected and in place of the beneficial associative bacteria, harmful types now predominate in the rhizosphere. Therefore, more

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attention being paid to the search for early root colonizers which directly or indirectly influence plant growth and productivity. Rhizobacteria from the rhizosphere and rhizoplane of healthy chickpea (*Cicer ariectinum* L.) plants with growth-promoting and pathogenic-suppressive abilities were isolated and identified as species of *Pseudomonas* and *Bacillus* (Parmar and Dadarwal, 1999).

The use of mixed biofertilizers is advocated to get the maximum benefits due to additive and synergistic effect. The role of symbiotic nitrogen fixing bacteria, Plant Growth Promoting Rhizobacteria (PGPR) and phosphate solubilizing microorganisms in crop productivity is well documented (Kennedy *et al*., 2004; Hariprasad and Niranjana, 2009). The PGPR play a crucial role in soil health and plant growth and have been used for biocontrol of plant pathogens. But the studies regarding coinoculation of *Mesorhizobium* sp. with phosphate solubilizer or/and PGPR in the presence/absence of inorganic fertilizers under field conditions are scarce. Co-inoculation studies with PGPR and *Rhizobium*, *Bradyrhizobium* sp. have shown to increase root and shoot weight, plant vigor, nitrogen fixation and grain yield in various legumes (Valverde *et al*., 2006; Yadegari *et al*., 2008). Phosphate-solubilizing *Bacillus* sp. stimulates plant growth through enhanced P nutrition and increasing the uptake of N, P, K and Fe (Biswas *et al*., 2000). Combined inoculation of *Rhizobium* with *Pseudomonas striata* or *Bacillus polymyxa* and with *Bacillus megaterium* have shown increased dry matter, grain yield and phosphorus uptake significantly over the uninoculated control in legumes (Elkoca *et al*., 2008).

Chickpea (*Cicer arietinum* L.) is a major grain legume crop important pulse crop. It is contributes to 38% of national pulse production in India. Chickpea can obtain a significant portion of its N requirement through symbiotic N_2-fixation to give high grain yield when grown in association with effective and competitive *Rhizobium* strain (Stephen *et al*., 2002). Interactions between these PGPR with *Rhizobium* may be antagonistic or synergistic and the beneficial effects of such interactions could be exploited for economic grain (Dubey, 1996). The objective of the present study was evaluation of chickpea (*Cicer aritenium* L.) seed inoculation with indigenous *Rhizobium* strain and plant growth promoting rhizobacteria on nodulation, plant growth and yield.

MATERIALS AND METHODS

Culture, Media and Growth Condition

The pure cultures of *Azotobacter chroococcum* strain MTCC-446 was obtained from MTCC (microbial technology culture collection), Institute of Microbial Technology, Chandigarh, Punjab, India. *Pseudomonas fluorescens* strain BHUPSB06 and *Bacillus megaterium* strain BHUPSB14 was isolated from rhizosphere soil of eastern Uttar Pradesh region at September, 2006. *Rhizobium* sp. BHURC01 was isolated from root nodules of chickpea plant from Eastern Uttar Pradesh. *Pseudomonas fluorescens* strain BHUPSB06, *B. megaterium* strain BHUPSB14 and *Rhizobium* sp. strain BHURC01 were characterized by biochemical and molecular methods. 16S rDNA gene sequencing had been done and sequence deposited in NCBI with different accession number GU124814 (*P. fluorescens* strain BHUPSB06), GU124821 (*B. megaterium* strain BHUPSB14) and GU124816 (*Rhizobium* sp. strain BHURC01). The bacterial strains of *A. chroococcum*, *P. fluorescens* and *B. megaterium* were maintained on nutrient agar medium. *Rhizobium* sp. strain BHURC01 was maintained on Yeast Extract Mannitol Agar (YEMA) medium. The pure fungal culture of *Fusarium oxysporum* and *Rhizoctonia solani* were obtained from Department of Plant Pathology and Mycology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The culture were maintained by periodic transfer and stored in the refrigerator for further studies.

In vitro Screening of Bacterial Strain for their Plant Growth Promoting Activities

The IAA (indole-3-acetic acid) production was estimated by the modified method as described by Brick *et al.* (1991). Phosphate solubilization was screened on Pikovskaya's agar plates as described by Gaur (1990). NH_3 production was tested in peptone water by the method of Cappuccino and Sherman (1992). Siderophore production was analyzed on the Chrome azurol S agar medium (Sigma, Pvt. Ltd.) as described by Schwyn and Neilands (1987). The HCN production was determined on nutrient agar plates supplemented with glycine (4.4 g L^{-1}) as described by Jha *et al.* (2009). Antifungal assay was determined by the method of Rajendran *et al.* (2008).

Host Seeds

Seeds of chickpea (*Cicer arietinum* L.) cultivar (C-235) were obtained from Indian Institute of Pulse Research (IIPR), Kalyanpur, Kanpur, Uttar Pradesh, India.

Seed Bacterization

The *Rhizobium* sp. BHURC01 was grown in YEM broth and *A. chroococcum*, *B. megaterium* and *P. fluorescens* were grown in Nutrient broth by incubation for 120 rpm at 28±2°C for 48 h. Healthy seeds weighed for each plot of 5 m^2 (at 100 kg ha^{-1}) were separately inoculated as per treatments in plastic bags with 5 mL of 7 days old broth cultures grown in specific media of respective inoculants (mixed in 1:1 ratio for combined treatments) along with 1 mL of 1% (w/v) sticker solution of gum acacia to ensure bacterial population in the range of 10^7 to 10^8 colony forming unit (cfu) $seed^{-1}$. After drying for 1 h in shade, uninoculated seeds were sown first followed by inoculated seeds just to avoid contamination.

Field Experiments

The field experiments were set up in the first week of October 2006 to March, 2007 (first-year experiment) and October 2007 to March, 2008 (second-year experiment) at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi Uttar Pradesh, India. The field experiments were conducted with 5 treatments and 3 replication of indigenous *Rhizobium* sp. strain BHURC01 and appropriate synergistic combination of Plant Growth Promoting Rhizobacteria (PGPR) as (*A. chroococcum* *P. fluorescens* and *B. megaterium*) and one uninoculated control. The plot size was 10×2 m and spacing 25×25 cm between row and 10×10 cm between plants. The physico-chemical properties of the initial soil of experimental field was sandy clay loam in texture with 40.83% water holding capacity, neutral in reaction (pH 7.25) and electronic conductivity (dS m^{-1}) 0.155. The content of organic carbon (0.778%) (Walkley and Black, 1934), available N (213.24 kg ha^{-1}) (Subbiah and Asija, 1956), P_2O_5 (27.22 kg ha^{-1}) (Olsen *et al.*, 1954) and K_2O (254.76 kg ha^{-1}) (Jackson, 1973), respectively in soil. The microbial population of total bacteria, fungi and actinomycetes (4.5×10^{-8}, 3.1×10^{-8} and 3.4×10^{-8} cfu g^{-1} soil), respectively.

Assessment of Nodules Number, Dry Weight of Root and Shoot, Yield

For assessment of root shoot dry weight, ten plants were randomly uprooted at flowering time (80 days after sowing) from inoculated and control plots and washed several time with running water. First roots were cut off and root and shoots were dried at 70°C for 72 h. separately, for each plant. For nodules number, another ten plants were uprooted from control and inoculated plots. The nodules were separated and counted from each plant and dry weight was recorded after drying the nodules at 70°C for 72 h. The ends of the growth

period, another ten plants were randomly harvested from control and inoculated plots to determine yield parameters. The plants were dried at 70°C for 72 h and weighted for calculating the biological yield. Grain weight was also recorded at the time of harvesting.

Determination of Nitrogen and Phosphorus in Nodules, Grain and Straw

For determination of nitrogen and phosphorus in nodules, ten representative plants from control and inoculated plots at flowering time were taken. The dry nodules (0.2 g), grains (0.2 g) and straw (0.5 g) samples were digested in 10 mL of 4.1 ratio of HNO_3: $HClO_4$ for total P (Vanadomolybdophosphoric acid yellow color methods) (Jackson, 1967) in 10 mL diacid mixture of 9:1 ratio of H_2SO_4: $HClO_4$ for the analysis of total N (Nessler's reagent method) (Jackson, 1973). Protein content in grain was calculated according to the formula: nitrogen (%) ×6.25 (conversion factor).

Experimental Design

The experiment was arranged in a randomized block design and was replicated four times. Statistical analysis was conducted using One-Way Analysis of Variance (ANOVA). Comparisons of mean were performed by the Least Significant Deferent (LSD) test at $p \leq 0.05$ by using SPSS software version 12.0.

RESULTS AND DISCUSSION

***In vitro* Screening Test of Bacterial Strain for their Plant Growth Promoting Activity**

Rhizobium sp. strain BHURC01, *P. fluorescens* BHUPSB06, *A. chroococcum* and *B. megaterium* BHUPSB14 were found to be positive for ammonia, IAA production (Table 1). Only *P. fluorescens* BHUPSB06 was found to be positive for HCN, siderophore production and positive for inhibition of growth of soil born phytophatogen (*Fusarium oxysporum* and *Rhizoctonia solani*). Tryptophan is a precursor of IAA biosynthesis. *Pseudomonas fluorescens* showed significant increase concentration of IAA as compared to other PGPR strains when tryptophan was added as the precursor. In broth, IAA production in bacterial culture varies from 35.11 to 114.19% at 100 μg mL^{-1} tryptophan after 72 h incubation. IAA production showed more significant in *P. fluorescens* (114.19%), *Rhizobium* sp. (70.37%) and *A. chroococcum* (35.11%) as compare with *B. megaterium* at 100 μg mL^{-1} tryptophan after 72 h incubation. Phosphate solubilization was most frequently encountered by *P. fluorescens* followed by *B. megaterium* least by *Rhizobium* sp. and *A. chroococcum*. *Pseudomonas fluorescens* produces largest halos, 20 mm (approx.) around their colonies within 5 days of incubation than others isolates. *Bacillus megaterium* produces 15 mm (approx.) halo zone around colonies on Pikovaskaya medium. However, production of ammonia was a common trait in all isolates of bacteria. Experimental results

Table 1: *In vitro* screening test of bacterial strain for their plant growth promoting activity

Bacterial strain	IAA (μg mL^{-1})	PSB (Halo zone mm)	NH_3	Siderophore	HCN	Mycelium growth inhibition	
						F. oxysporum	*R. solani*
Rhizobium sp.	12.13±1.23[a]	4	+	-	-	-	-
A. chroococcum	7.62±1.13[b]	5	+	-	-	-	-
P. fluorescens	15.25±1.51[c]	20	+	+	+	+	+
B. megaterium	3.10±0.12[d]	15	+	-	-	-	-

+: Positive, -: Negative test, PSB: Phosphate solubilizing Bacteria; NH_3: Ammonia production; HCN: Hydrogen cyanide, IAA-Indole Acetic Acid (μg mL^{-1}±SD). Data are average values of three replicates±SD. Mean with different letter(s) in the same column differ significantly at $p \leq 0.05$ according to Fisher's Protected LSD

Table 2: Effect of Plant Growth Promoting Rhizobacteria (PGPR) and *Rhizobium* sp. BHURC01 inoculation on nodule related parameters of chickpea in field experiments of two years

Treatments	Nodule No. plant^{-1}	Nodule dry weight (g) plant^{-1}	Nitrogen % in nodule	Phosphorus % in nodule
Year 2006-2007				
Control	40±2.12^{a}	0.128±0.007^{a}	3.48±0.44^{a}	0.151±0.001^{a}
Rhizobium sp.	52±1.21^{b}	0.152±0.011^{ab}	4.12±0.41^{a}	0.158±0.001^{b}
Rhizobium sp. +*A. chroococcum*	58±2.17^{c}	0.163±0.03^{ab}	3.97±0.28^{a}	0.162±0.002^{c}
Rhizobium sp. +*P. fluorescens*	68±1.61^{d}	0.185±0.02^{b}	4.85±0.25^{b}	0.172±0.004^{d}
Rhizobium sp. +*B. megaterium*	62±1.62^{c}	0.160±0.03^{b}	3.85±0.12^{a}	0.168±0.002^{b}
Year 2007-2008				
Control	47±1.5^{a}	0.125±0.013^{a}	3.01±0.03^{a}	0.141±0.001^{a}
Rhizobium sp.	58±0.51^{b}	0.156±0.01^{b}	3.25±0.41^{b}	0.162±0.02^{a}
Rhizobium sp. +*A. chroococcum*	62±2.8^{c}	0.158±0.005^{b}	4.35±0.27^{c}	0.159±0.003^{a}
Rhizobium sp. +*P. fluorescens*	79±2.6^{d}	0.172±0.002^{c}	4.45±0.29^{bd}	0.168±0.001^{b}
Rhizobium sp. +*B. megaterium*	72±1.6^{e}	0.164±0.001^{c}	4.12±0.48^{b}	0.162±0.023^{a}

Values are the Mean±SD. Mean values in each column with the same letter(s) do not differ significantly by LSD (p=0.05)

revealed that *P. fluorescens* is capable of siderophore and HCN production; where as other strains were not. *Pseudomonas fluorescens* also showed mycelial growth inhibition against *Fusarium oxysporum* and *Rhizoctonia solani*.

Effect of Plant Growth Promoting Rhizobacteria (PGPR) and *Rhizobium* sp. BHURC01 Inoculation on Growth of Nodule

The three PGPR isolates (*A. chroococcum*, *P. fluorescens* and *B. megaterium*) did not antagonize *Rhizobium* sp. BHURC01 when grown together on plates. The *Rhizobium* sp. BHURC01 interacted differentially with PGPR isolates and showed significant variation in nodulation, dry weight of nodules (Table 2). Dual inoculation of seed with *Rhizobium* sp. BHURC01 and *P. fluorescens*, *B. megaterium* and *A. chroococcum* were produced 30.77, 19.23 and 11.54%, respectively, more nodule number in first year experiment and 36.21, 24.14 and 6.90%, respectively, more nodule in second year experiment than *Rhizobium* strain inoculation alone. The maximum significant increased nodule dry weight 44.53 and 37.6%, nitrogen 39.37 and 47.84% and phosphorus in nodules 13.91 and 19.15% in the first and second year study, respectively in combination of *Rhizobium* sp. BHURC01 and *P. fluorescens*, followed by combination of *Rhizobium* sp. BHURC01, *B. megaterium* and *A. chroococcum* over uninoculated control.

Effect of PGPR and *Rhizobium* sp. BHURC01 Inoculation on Biomass Production and Yield

Table 3 shows that co-inoculation of seed with *Rhizobium* sp. BHURC01 and *P. fluorescens* showed significant increase dry weight of root 23.66 and 30.23% in first and second year of field study, respectively followed by combination of *Rhizobium* sp. BHURC01, *B. megaterium* and *A. chroococcum* as compare with uninoculated control. Similarly, shoot dry weight was more increased significant, 46.56 and 79.46% in the first year and second year of field experiment, respectively. The higher grain yield 37.26 and 23.64% and straw yield 27.95 and 21.70% in first and second year of field study, respectively was increased significantly in co-inoculation of *Rhizobium* sp. BHURC01 with *P. fluorescens* followed by *B. megaterium* and *A. chroococcum* over uninoculated control (Table 3).

Effect of PGPR and *Rhizobium* sp. BHURC01 Inoculation on Nitrogen and Phosphorus Content in Grain and Straw and Grain Protein

The maximum nitrogen in grain 31.75 and 39.67% and in straw 55.17 and 48.38% in first and second year of study, respectively, while maximum significant phosphorus in grain

Table 3: Effect of PGPR and *Rhizobium* sp. BHURC01 inoculation on biomass production and yield-related parameters of chickpea in field experiments of two years

	Biomass production (Dry weight g plant^{-1})		Yield (q ha^{-1})	
Treatments	Root	Shoot	Grain	Straw
Year 2006-2007				
Control	0.131±0.001^{a}	1.13±0.13^{a}	20.1±1.26^{a}	14.31±0.79^{a}
Rhizobium sp.	0.142±0.002^{a}	1.38±0.31^{a}	23.81±0.83^{b}	15.23±0.35^{a}
Rhizobium sp. +*A. chroococcum*	0.151±0.003^{b}	1.85±0.07^{b}	26.21±0.94^{c}	17.24±0.75^{b}
Rhizobium sp. +*P. fluorescens*	0.162±0.004^{b}	1.92±0.03^{b}	27.59±0.18^{d}	18.31±0.65^{b}
Rhizobium sp. +*B. megaterium*	0.158±0.004^{b}	1.78±0.05^{b}	24.82±0.94^{e}	16.89±0.59b
Year 2007-2008				
Control	0.129±0.003^{a}	1.12±0.09^{a}	21.15±1.09^{a}	15.21±0.06^{a}
Rhizobium sp.	0.147±0.01^{b}	1.421±0.09^{b}	23.08±0.81^{b}	16.82±0.42^{bc}
Rhizobium sp. +*A. chroococcum*	0.151±0.01^{b}	1.72±0.11^{d}	24.12±1.52^{c}	17.15±1.71^{bc}
Rhizobium sp. +*P. fluorescens*	0.168±0.001^{c}	2.11±0.02^{e}	26.15±0.85^{c}	18.51±1.51^{c}
Rhizobium sp. +*B. megaterium*	0.156±0.002^{c}	1.89±0.11^{cd}	25.21±0.87^{c}	17.61±1.41^{c}

Values are the Mean±SD. Mean values in each column with the same letter(s) do not differ significantly by LSD (p=0.05)

Table 4: Effect of PGPR and *Rhizobium* sp. BHURC01 inoculation on nitrogen and phosphorus content in grain and straw of chickpea in field experiments of two consecutive years

	Grain (%)		Straw (%)		Grain (%)
Treatments	Nitrogen	Phosphorus	Nitrogen	Phosphorus	Protein
Year 2006-2007					
Control	3.15±0.06^{a}	0.36±0.01^{a}	0.29±0.02^{a}	0.111±0.001^{a}	19.15±1.23^{a}
Rhizobium sp.	3.36±0.25^{a}	0.43±0.01^{a}	0.36±0.01^{b}	0.131±0.021^{b}	20.10±1.54^{a}
Rhizobium sp.+*A. chroococcum*	3.56±0.23^{a}	0.45±0.13^{a}	0.38±0.05^{b}	0.135±0.005^{b}	21.15±0.54^{b}
Rhizobium sp. +*P. fluorescens*	4.15±0.18^{b}	0.55±0.05^{b}	0.45±0.01^{c}	0.151±0.018^{c}	23.01± 0.28^{c}
Rhizobium sp. +*B. megaterium*	3.32±0.45^{a}	0.46±0.01^{b}	0.40±0.06^{b}	0.145±0.009^{d}	22.11±0.78^{b}
Year 2007-2008					
Control	3.05±0.06^{a}	0.38±0.05^{a}	0.31±0.01^{a}	0.112±0.01^{a}	19.25±1.24^{a}
Rhizobium sp.	3.76±0.15^{b}	0.41±0.03^{a}	0.41±0.01^{b}	0.135±0.01^{b}	22.01±0.26^{b}
Rhizobium sp. +*A. chroococcum*	3.65±0.21^{b}	0.41±0.14^{a}	0.39±0.01^{b}	0.142±0.02^{bd}	23.01±0.45^{b}
Rhizobium sp. +*P. fluorescens*	4.26±0.29^{cd}	0.51±0.02^{b}	0.46±0.01^{c}	0.149±0.01^{c}	24.15±1.41^{c}
Rhizobium sp. +*B. megaterium*	3.85±0.25^{d}	0.45±0.03^{a}	0.36±0.02^{b}	0.146±0.01^{cd}	22.15±1.34^{b}

Values are the Mean±SD. Mean values in each column with the same letter(s) do not differ significantly by LSD (p=0.05)

52.78 and 34.21% and in straw 36.04 and 33.04% in inoculation of *Rhizobium* sp. BHURC01 and *P. fluorescens* over uninoculated control (Table 4). The protein content in grain was also increased significant 20.16 and 25.45% at first and second year of field study, respectively in co-inoculation of *Rhizobium* sp. BHURC01 with *P. fluorescens* followed by *B. megaterium* and *A. chroococcum* over uninoculated control.

DISCUSSION

The IAA production showed significant increase in *P. fluorescens*, *Rhizobium* sp. and *A. chroococcum* as compare with *B. megaterium*. Similar high level of IAA production was recorded in *Pseudomonas* by Ahmad *et al.* (2008). Wani *et al.* (2007) have been reported *Rhizobium* sp. increased IAA production by 79, 98 and 152% over *Bacillus* PSB1, *Bacillus* PSB10 and *A. chroococcum* A4, respectively. Generally, the IAA production increased with increasing tryptophan. Moreover, it has been reported that IAA production by Plant Growth Promoting Rhizobacteria (PGPR) can vary among different species and strains and that it is also influenced by culture condition, growth stage and substrate availability (Beneduzi *et al.*, 2008). According to De Freitas *et al.* (1997) good phosphate-solubilizers were able to produce halos of more than 15 mm diameters around their colonies. Several species of fluorescent pseudomonas such as *P. fluorescens* NJ101 (Bano and Musarrat, 2004),

P. aeruginosa (Jha *et al*., 2009) and *Bacillus* sp. (Ahmad *et al*., 2008) were reported as good phosphate solubilizers. However, production of ammonia was a common trait in all isolates of bacteria. *Pseudomonas fluorescens* was detected positive for siderophore and HCN production than other strains of plant growth promoting bacteria. *Pseudomonas fluorescens* prevents the wilting and root rot disease in chickpea plant by inhibiting the growth of soil pathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani* than other strains under culture condition. In this study, *P. fluorescens* was found to produce HCN. The HCN affects the respiratory system of pathogenic fungi and results in their growth inhibition (Kirimura *et al*., 1987). Several studies have demonstrated that production of siderophores, other secondary metabolites and lytic enzymes by pseudomonas strains was most effective in controlling the plant root pathogens including *F. oxysporum* and *R. solani* (Nagrajkumar *et al*., 2004; Ahmad *et al*., 2008).

Co-inoculation studies with PGPR and *Rhizobium*, *Bradyrhizobium* sp. have been shown to increase root and shoot biomass, nodule dry matter, N_2-fixation and grain yield in chickpea (Gull *et al*., 2004) and various legume such as common bean green gram (Sindhu *et al*., 1999) and pigeonpea (Tilak *et al.,* 2006). Furthermore, combined inoculations with N_2-fixing and P-solubilizing bacteria were more effective than single inoculation possibly by providing a more balanced nutrition for plants (Belimov *et al*., 1995). Over all maximum significant result have been shown in combination of *Rhizobium* sp. BHURC01 with *P. fluorescens* followed by other combination as *Rhizobium* sp. strain with *B. megaterium* and *A. chroococcus* over uninoculated control in two year of field experiment. Co-inoculation of *Rhizobium* sp. and *P. fluorescens* has been found more significant for chickpea production because *Rhizobium* sp. strain is an effective nitrogen fixer and *P. fluorescens* is an effective PGPR than other bacterial strains. Direct mechanism of PGPR has stimulated plant growth by the production of phytohormone (IAA), improved nutrient acquisition increasing crop nutrient uptake of N from nitrogen fixing bacteria (*Rhizobium* sp.) and uptake of P from phosphate mineral solubilizing bacteria (*P. fluorescens* and *B. megaterium*) and uptake of iron from siderophore producing bacteria (*P. fluorescens*). Indirect mechanism of PGPR has suppressed plant diseases like wilt disease (*Fusarium oxysporum*) and root rot (*Rhizoctonia solani*) by *Pseudomonas fluorecsens*. Thus, the present finding supports that a composite application of symbiotic N_2-fixing organisms and plant growth promoting rhizobacteria could improved plant growth and nutrient uptake, leading to significant increases in the grain yield and protein content of field-grown chickpea. Wani *et al.* (2007) have been reported the synergistic effect of nitrogen fixing and phosphate-solubilizing rhizobacteria on plant growth, yield, grain protein and nutrient uptake of chickpea plants. That all of the bacterial inoculations resulted in considerable yield increases compared with control and co-inoculations, especially microbial fertilization could be an alternative to NP fertilization in chickpea (*Cicer arietinum* L.) (Elkoca *et al*., 2008; Rudresh *et al*., 2005).

CONCLUSION

In over all study of two year field experiment data, we have been found synergistic combination of *Rhizobium* sp. BHURC01 and *P. fluorescens* for better growth and yield of chickpea production. The potential of these rhizobacteria to inhibit the growth of the phytopathogenic fungi leading to suppression of the plant disease, along with their nodule promoting and plant growth promoting effects on co-inoculation with *Rhizobium* sp. BHURC01 suggested that these bacteria could be exploited to improve chickpea (*Cicer arietinum* L.) production.

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